

Yogurt Fermentation in the Presence of Starch–Lipid Composite

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ABSTRACT: The fermentation of yogurt in the presence of 0.5%, 1.0%, 1.5%, and 2.0% starch–lipid composite (SLC) was investigated. The pH, viscosity, and morphology of the mix were monitored during the fermentation process. The rate of drop in pH with time during incubation was not affected by the addition of SLC. However, it was found that the presence of SLC caused faster aggregation, which was clearly evidenced by the viscosity variation during the process of fermentation. An examination of the morphologies confirmed that aggregation occurred earlier in the presence of SLC and SLC did not form phase-separated domains. This study concludes that SLC would serve as a good additive (fat replacer and stabilizer) for the production of yogurt.

Keywords: aggregation, fermentation, gelling, viscosity, yogurt

Introduction

Yogurt is made by fermenting liquid milk. The conversion of milk to yogurt is aggregation of casein micelles into a gel structure of 3-dimensional networks at low pH. The pH is lowered by specific fermentation with bacteria that convert milk sugar (lactose) to lactic acid in the yogurts (Tamime and Marshall 1977). The pH can also be lowered chemically by direct addition of mineral acids to form acid casein gels (Arshad and others 1993; Cobos and others 1995; Mulvihill and Grufferty 1995). Various nondairy ingredients such as starch, whey protein concentrates, gelatin, and hydrocolloids have been added to yogurt as fat replacers and to modify the rheological properties (Mistry and Hassan 1992; Keogh and O’Kennedy 1998; Guzmán-González and others 1999; Decourcelle and others 2004; Sodini and others 2004). The addition of whey protein concentrates can lead to powdery taste, excessive acid development, excessive firmness, higher syneresis, and grainy texture (Mistry and Hassan 1992; Guzmán-González and others 1999). The addition of starch increases the viscosity of yogurt, but some starches impart an undesirable taste and promote phase separation (Williams and others 2004).

Starch–lipid composites (SLCs) are stable emulsions formed by jet-cooking starch under excess steam and adding fat to it (Fanta and Eskins 1995). Microscopic analyses have shown that composites consist of 1-to 10- μ m-dia lipid droplets coated with a thin film of firmly bound starch at the lipid–water interface (Eskins and Fanta 1996; Fanta and others 1999). Starch in SLC provides the functionality, while the lipid fraction acts as a carrier of desirable flavors and adds to the creamy mouthfeel in the products. The addition of SLC resulted in no syneresis for yogurt samples stored for 3 wk at 4 °C (Singh and Byars 2008).

The objective of this study was to examine the suitability of SLC as an additive to the mix for the yogurt formation. For that purpose,

pH, viscosity, and morphology of yogurt mixes with and without SLC were monitored during fermentation.

Materials and Methods

Starch–lipid composites (SLCs)

Starch (food-grade dent corn) was mixed with water to a solids content of 25.6%. The slurry was stirred in a Waring blender (Model 37BL84; Dynamics Corp. of America, New Hartford, Conn., U.S.A.) and pumped through a laboratory model steam jet cooker consisting of a progressive cavity pump (Robins and Myers Inc., Springfield, Ohio, U.S.A.) and manual stainless steel hydroheater (Hydrothermal, Waukesha, Wis., U.S.A.). The jet cooker was operated under excess steam conditions. The outlet pressure was maintained at 380 kPa (140 °C) and the steam line pressure was 550 kPa (155 °C). The pumping rate was 1 L/min. Melted butter (unsalted sweet butter; Land O’Lakes, Arden Hills, Minn., U.S.A.) was added to the resulting dispersion in a 1:10 lipid:starch ratio (w/w) and passed through the jet cooker again under the conditions described above. Butter was chosen as the lipid source in the composite to disperse natural milk fat flavor in the yogurt. The resulting SLC was dried on a double-drum drier (Model 20; Drum Drier and Flaker Co, South Bend, Ind., U.S.A.) heated with steam at 310 kPa (135 °C). The flaky product was ground to a coarse powder in a Retsch mill (type ZM1; Brinkmann Instruments, Inc., Westbury, N.Y., U.S.A.), packed in polyethylene bags, and refrigerated until use.

Yogurt mix preparation

The yogurt mixes were prepared by blending in 9% (w/w) non-fat milk solids (carnation instant nonfat dry milk; Nestle USA Inc., Solon, Ohio, U.S.A.) with fluid skim milk at 50 °C. SLC (0%, 0.5%, 1%, 1.5%, and 2%) was added to the mix with dry milk solids and blended with a hand blender for 1 min. Mixes were heated to 90 °C in a water bath and held for 30 min, and then rapidly cooled to 40 °C in an ice bath. Freeze-dried yogurt culture containing *Streptococcus thermophilus*, *Lactobacillus delbrueckii* subspecies *bulgaricus*, *Lactobacillus acidophilus*, and *Bifidobacterium* (Yo-Fast 88; 200U; Chr. Hansen, Milwaukee, Wis., U.S.A.) was used at the rate of 1 U/L. Stock culture was prepared by dissolving the freeze-dried culture in MilliQ water to a concentration of 1 U/mL. The fermentation of each yogurt batch was performed at 40 °C.

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Morphology of yogurt during fermentation

The fermentation process of yogurt was monitored by both phase-contrast and fluorescence microscopy. The development of agglomeration in the sample specimens was observed by placing a drop of solution in between a glass slide and a cover slip. Microscopic images were obtained with an inverted microscope (Zeiss; Merz Optical Instruments, Chicago, Ill., U.S.A.). Both phase contrast image and fluorescence image were taken by attaching a digital camera (Model D100; Nikon Corp., Tokyo, Japan) through a relay lens. The obtained images were transferred to a Mac computer and processed with image software (Photoshop v. 7.0; Adobe Systems Inc., San Jose, Calif., U.S.A.) for optimum brightness/contrast control.

Viscosity and pH

Yogurt pH was measured using an Accumet X 150 pH meter equipped with an ATC probe for temperature effect correction. The pH of the inoculated yogurt mix was recorded every 2 min during fermentation.

Viscosity measurement was performed with a DV-III Ultra rheometer (Brookfield Engineering Laboratories, Inc., Middleboro, Mass., U.S.A.) equipped with a small sample adapter with a spindle (nr SC4-31). The viscosity data of the samples were taken every minute. To minimize the stirring effect by the rotating spindle, a very low shear rate (10 per second) was applied for 5 s only when data were taken. The temperature of the mix for pH and viscosity measurements was set to 40 ± 0.1 °C with a circulating bath (Type K2-R; Lauda Thermostat, Lauda, Germany) throughout the experiment.

Labeling yogurt

7-Chloro-4-Nitrobenz-2-Oxa-1,3-Diazole (NBD-Cl) is widely used to label peptides, proteins, drugs, and other biomolecules (Lundblad 2005). NBD-Cl is nonfluorescent, but generates fluorescent products upon reacting with amino groups such as aliphatic amines, amino acids, peptides, and proteins to form highly fluorescent compounds. Fluid milk, 390 g, was mixed with 130 mg of NBD-Cl (Sigma Chemical Co; St. Louis, Mo., U.S.A.), and stirred for a day at room temperature. The progress of the reaction was easily identified by the development of fluorescence of the solution with time. The reaction was performed for 24 h at which time there was no further change in color. If we assume that all the dye molecules are reacted, the highest possible labeling ratio would be about 1 mole percent of the proteins in fluid milk. For the preparation of the yogurt samples, 30% of the fluid milk was replaced with the labeled milk.

Statistical analysis

Pearson correlation coefficients were calculated using the correlation procedures of the SAS, version 9.1 (SAS Inst Inc., Cary, N.C., U.S.A.). Basic statistics were calculated and graphs plotted using the software Kaleidagraph version 4.0.

Results and Discussion

The drop in pH is indicative of the progress of fermentation of yogurt during the incubation period. The pH drops as the lactose in the mix is fermented to lactic acid by the bacteria. The record of pH change with time during the incubation of yogurt mixes with 0%, 0.5%, 1%, 1.5%, and 2% levels of SLC is presented in Figure 1. The fermentation process is described in 3 phases of pH change, the lag phase, logarithmic phase, and the slow-down of acidification phase (Soukoulis and others 2007). SLC did not affect the rate of yogurt fermentation by the bacteria in mixes as seen by the drop

in pH with time (Figure 1). These results are in agreement with the previous work done with the addition of starch (Williams and others 2003; Oh and others 2007).

Viscosity development is related to the aggregation of casein micelles and gel formation consequently to the biochemical and physicochemical changes during milk fermentation. The yogurt mix is fluid-like before fermentation. As the mix starts to form a gel the viscosity increases enormously due to aggregation. Therefore, we are able to detect the moment of aggregation by monitoring the viscosity of the mix during fermentation. As an example, the change in viscosity with time for a yogurt mix sample with 1% SLC is shown in Figure 2. The viscosity of yogurt mix was unchanged up to the onset of aggregation, and then it rapidly increased to yogurt viscosity. Yogurt fermentation is characterized as a lag phase (stationary viscosity) and a logarithmic phase (rapid viscosity development), followed by constant or slightly reduced viscosity (Shaker and others 2000, 2001; Tamime and Robinson 2007). Increase in

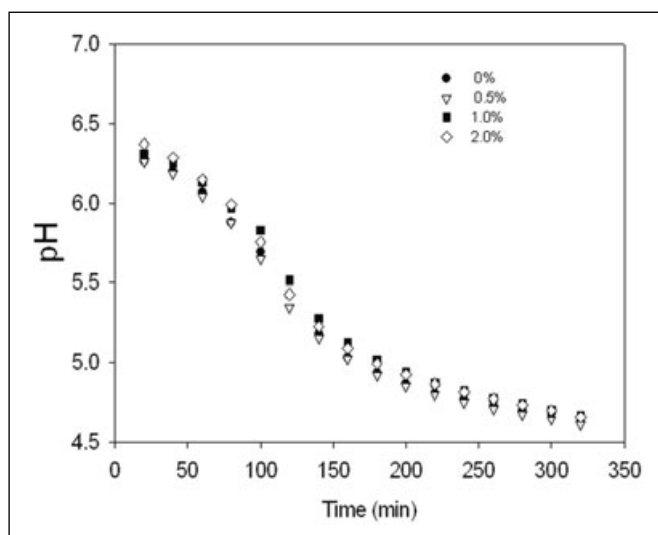


Figure 1—pH variation during the fermentation of yogurt mixes at various SLC levels (0%, 0.5%, 1%, 1.5%, and 2%).

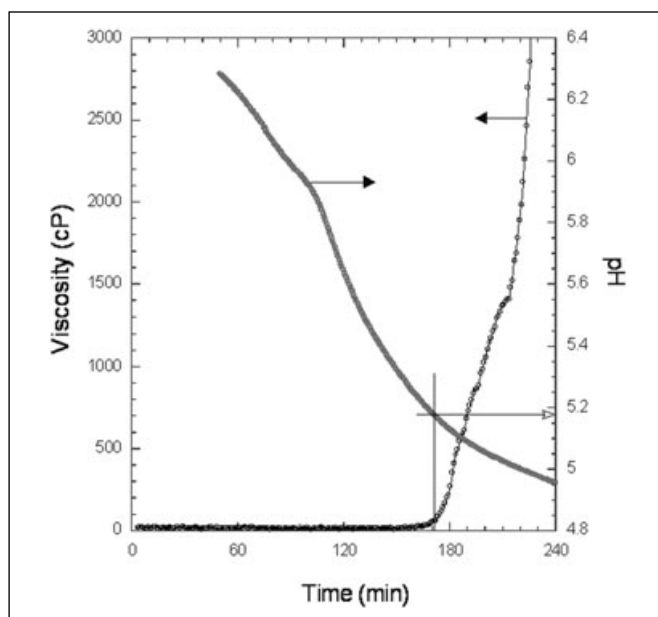


Figure 2—Viscosity and pH of yogurt mix for a sample with 1% SLC during the fermentation.

viscosity is an indication of casein-particle aggregation leading to gelation (Dagleish and Law 1988). In this study, onset of aggregation is defined as the start of the logarithmic phase for viscosity development as depicted in Figure 2. In the same figure, determination of onset time and onset pH is illustrated with a 1% SLC sample as an example. The aggregation of casein particles with acidification is considered as the result of 2 major destabilizing actions: the elimination of the steric repulsion inherent to the hairy layer and the neutralization of electrostatic repulsion (Banon and Hardy 1992).

The effect of amount of SLC on the aggregation time is shown in Figure 3. It is clearly shown that the addition of SLC induces ear-

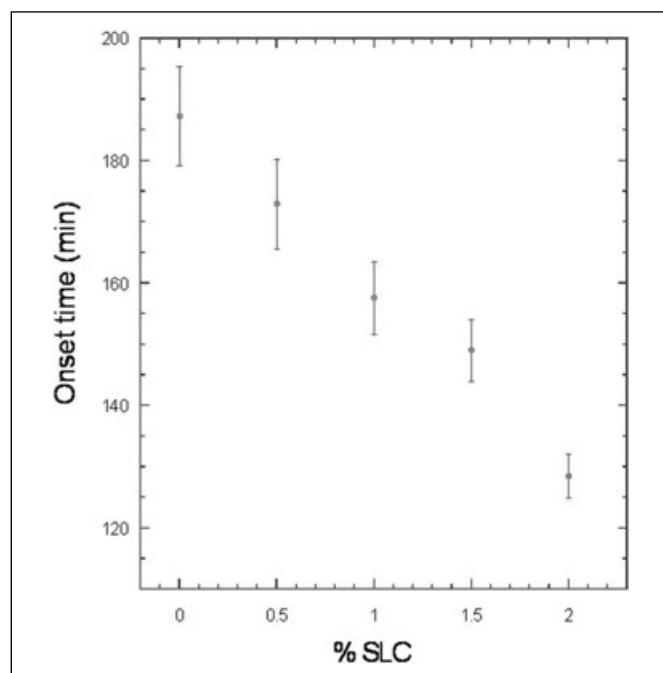


Figure 3—Effect of SLC on the onset time of aggregation of yogurt.

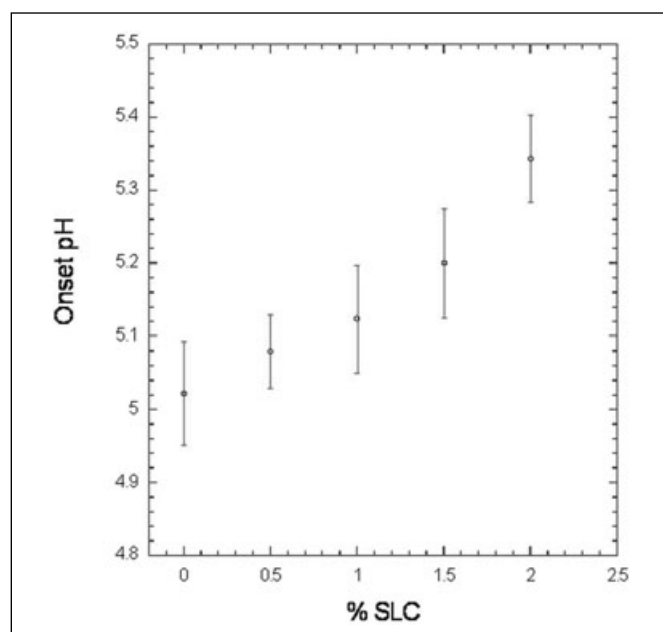


Figure 4—Effect of SLC levels on the onset pH of aggregation of yogurt.

lier onset of aggregation and aggregate formation gets faster with more added SLC. The onset time of aggregation ranged from 128 to 187 min. The onset time was significantly reduced with the addition of 2% SLC in the yogurt mix. On the other hand, the pH at the onset of aggregation was in the range of 5 to 5.4 with added SLC (Figure 4). A pH of 5.1 as onset point for set-style yogurt has been reported (Gastaldi and others 1996). Since it was already shown

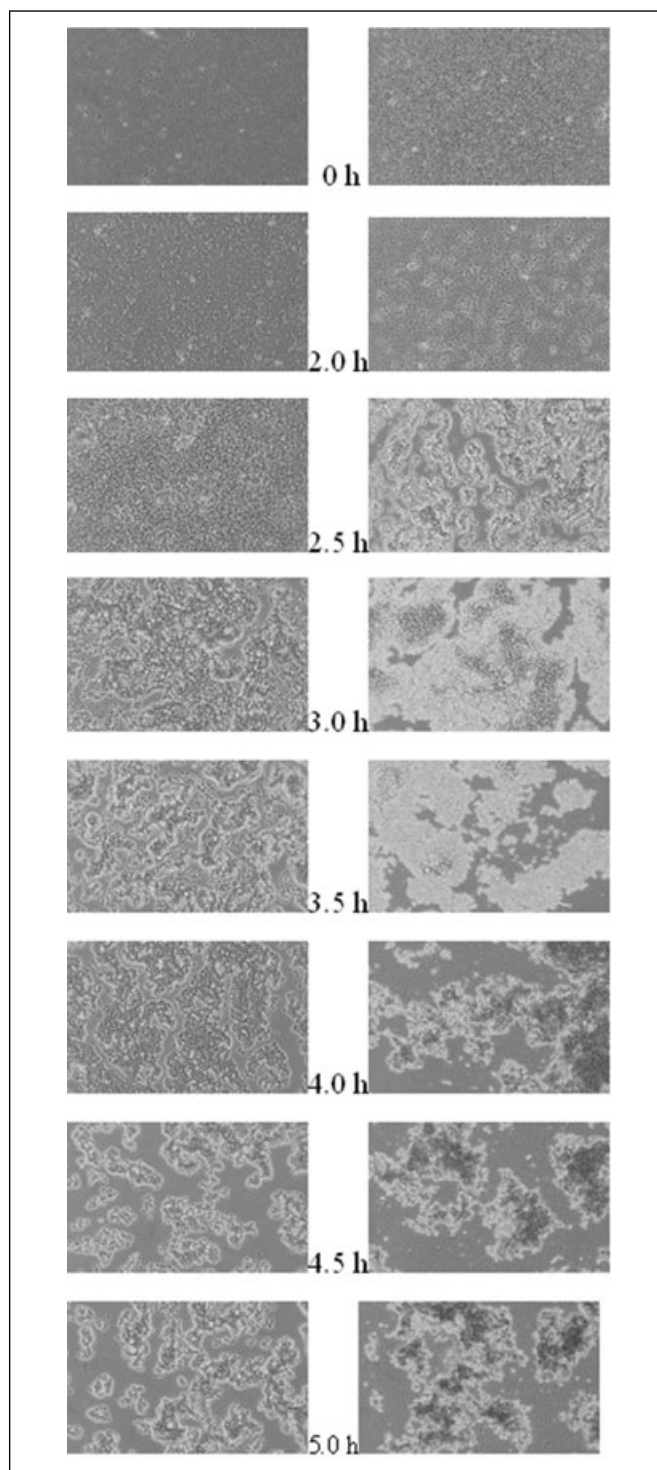


Figure 5—Phase contrast images of yogurt mix samples with 2% and without SLC during the fermentation process. Left column images are with no added SLC, and the right column images are with 2% added SLC. The abscissa is 770 μm .

that the addition of SLC did not change the incubation process (Figure 1), our experimental result shows that SLC readily induces aggregation at higher pH than 5.1 (our measurement is pH 5.0). The reason for this behavior is not understood yet. The earlier onset of aggregation with the addition of SLC was confirmed by the phase contrast images of yogurt mixes during fermentation.

The microstructure of yogurt has been described as a 3-dimensional network of chains and clusters of casein micelles retaining their globular shapes (Kalab and others 1975). The 3-dimensional network is large enough to be observed with an optical microscope. The evolution of the protein network structure was observed with a phase contrast microscope during the fermentation process of the yogurt mix (Figure 5). In the control, a fine dispersion of particles was observed. No distinct changes in the structure of control mix were observed till 1.5 h of fermentation. Starting at 1.5 h, and continuing for 2 h of fermentation, the suspended particles increased in size and aggregates started to form at 3 h with aggregation increasing in size to form gels. The images taken at initial stages of incubation do not show any significant differences leading to clear aggregation at the 4 h. Suspended particles were observed in the mix containing SLC. Aggregation at 2.5 h for 2% SLC is at the similar stage as that of control at 4 h. These observations confirm that the presence of SLC in the yogurt mix results in aggregation at an earlier time. A yogurt mix with 2% SLC showed distinct changes occurring between 2 and 2.5 h and also that once the aggregation started the rate of gelation was much faster. It indicates that aggregation is accelerated with the presence of starch and lipid. At the late stage of the fermentation, higher contrast images were observed under the phase-contrast microscope. It indicates that density of the aggregates was increased whereby the refractive index difference between aggregates and serum was increased. This result is supported by the report from Oh and others (2007) who observed an increase in the density of the protein network in the presence of starch. No phase separation was observed with the addition of the SLC. Oh and others (2007) also reported that with the

addition of starch the protein network remained as a continuous dominant phase. The phase contrast images taken during the development of yogurt gel (Figure 5) showed that agglomeration occurs at an earlier time and at a much faster rate with the addition of SLC. Since we could mistakenly regard the phase-separated SLC as protein aggregates, fluorescence microscopy was used to further investigate the fermentation process with added SLC. Phase contrast and fluorescence images of a sample with 2% SLC prepared with labeled milk after 3 h of fermentation are shown in Figure 6.

The image clearly shows that the aggregates formed were of milk protein origin only and there were no phase-separated domains. Thus it is concluded that SLC is embedded in the casein aggregates. In a previous study, it was observed that SLC strengthens the aggregates formed during the yogurt fermentation, forming a stronger gel, and resulting in decreased syneresis with storage (Singh and Byars 2008). Therefore, it is suggested that SLC reinforces the casein network in the yogurt gel.

Yogurt fermentation involves the conversion of lactose to lactic acid by bacteria, reducing the pH to 4.6. If lowering the pH of the yogurt mix is the major factor for the induction of aggregation, fermentation can be simulated by replacing the bacteria with addition of organic acid in the yogurt mix. The pH of yogurt mix without bacterial culture was gradually lowered by adding 8% acetic acid drop by drop to mimic the fermentation process. Repeated trials confirmed that the aforementioned assumption was correct. As an example, the aggregation of yogurt mix induced by acid in the vicinity of onset pH is visually depicted in Figure 7. The aggregation of casein occurred at pH 5.0 and the aggregates became denser at pH 4.9. This is in accordance with the data obtained from yogurt mix fermented with bacterial culture. This confirms that pH is a major factor that governs the aggregation of casein in yogurt fermentation. Others have also reported that acidification is a key mechanism during yogurt fermentation (De Brabandere and De Baerdemaeker 1999)

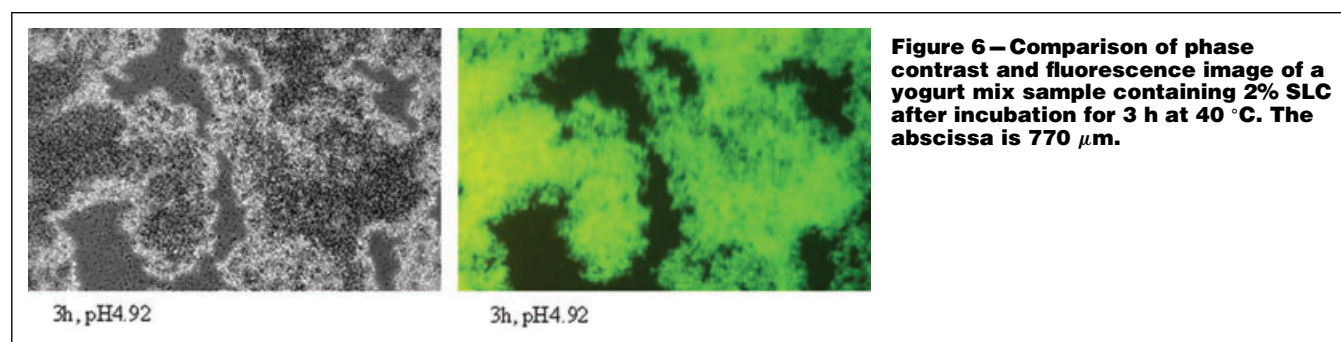


Figure 6 – Comparison of phase contrast and fluorescence image of a yogurt mix sample containing 2% SLC after incubation for 3 h at 40 °C. The abscissa is 770 μm .

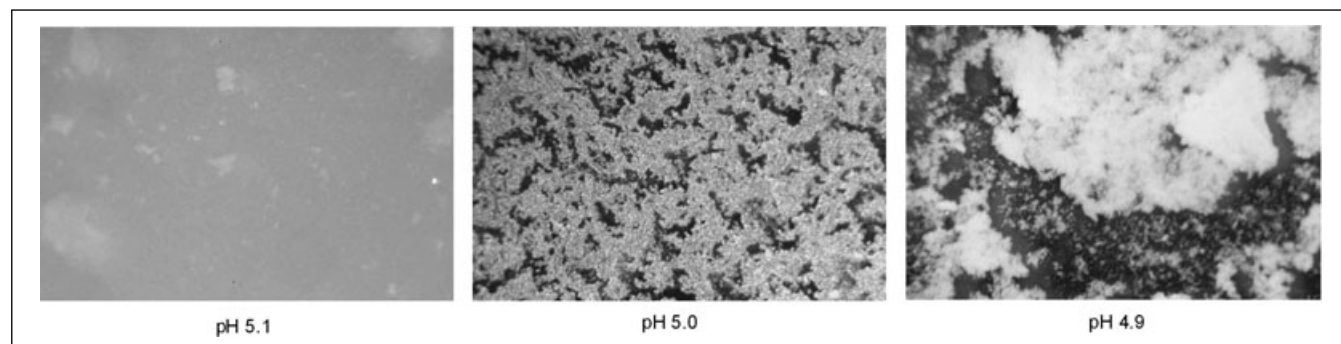


Figure 7 – Phase contrast images of acid-simulated milk gel depicting the onset of aggregation. The abscissa is 3000 μm .

To rule out the possibility that SLC did not participate in the aggregate formation during the fermentation, the supernatant obtained after centrifugation of yogurt with and without SLC was examined. If SLC stays in the liquid phase, the supernatant should be highly turbid. Regardless of the amount of added SLC, the supernatant was observed to be fairly clear indicating that SLC was part of the aggregates. Since the fluorescence microscopy experiment already showed that SLC did not form any phase-separated domains in the aggregates, it is concluded that SLC co-aggregated with the casein in the mix. As the aggregate formation was earlier with SLC, it is proposed that SLC served as nuclei for aggregate formation. Further work is needed to understand the specific interaction of SLC with casein during the fermentation process.

Conclusions

The SLC added to the yogurt mix does not interfere with the fermentation of lactose to lactic acid. SLC initiates increase in viscosity of the mix by aggregation at a higher pH. SLC co-aggregates with casein to form a stronger gel. SLC can be added to yogurt mix to increase the viscosity and to add creamy flavor and texture in low-fat yogurt products.

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